RAD DRAFT EXPOSURE REPORT (11/1/2018)

STANDARD REVIEW ENGINEERING REPORT [redacted]

MCAN: J18-0044 ASSESSOR: Lynch

SUBMITTER: Biosystems and Biomaterials Division
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MICROORGANISMS:

Recipient/host (p. 2):

The recipient microorganism is *Saccharomyces cerevisiae* BY4739 (MATalpha leu2Δ0 lys2Δ0 ura3Δ0) derived from *S. cerevisiae* 288C.

Donors (p. 4-5):

- URA3 gene from *M. jannaschii* DSM 2661 for selection of transformed cells; thus, the subject microorganism can grow in both typical yeast broth (YPD) or in yeast synthetic defined (SD) broth without uracil.
- DNA sequence (ERCC-00095) from *Methanocaldococcus jannaschii* to give specificity to the strain (detection marker).

GEM: J18-0044 is referred to as *Saccharomyces cerevisiae* NE095 (NIST ERCC 00095) (p. 1).

PV (CFU/yr) (p. 23):

Original Batch (Year 1):

$PV = (5.0 \times 10^7 \text{ CFU/mL})(4 \text{ L})(1000 \text{ mL/L})$
$PV = 2.0 \times 10^{11} \text{ CFU/yr}$ (1 batch/yr)

Potential 2nd Batch (Year 3):

$PV = (5.0 \times 10^7 \text{ CFU/mL})(12 \text{ L})(1000 \text{ mL/L})$
$PV = 6.0 \times 10^{11} \text{ CFU/yr}$ (1 batch/yr)

USE:

The majority (> 70 %) of the use is expected to be for training and exercises relevant to biological detection. The remaining ≈ 30 % of use cases are expected to focus on validation of microbial enumeration technologies. (p. 23-24).

SUMMARY:

The specific insertions/deletions (and corresponding effects) associated with the GEM are discussed in detail on pages 3-22 of the submission.

Submission states that all manufacturing in the next 3 years will occur at a toll site (likely Microbiologics, Inc. in St. Cloud, MN) (p. 23 and technical contact). Seed and main fermentors are used to grow the MCAN. Upon completion of the fermentation, the vials containing the GEM are sent to other sites for the downstream training and biological detections uses. The broth is inactivated through autoclaving or exposure to fresh sodium hypochlorite solution (10% by volume) for 20 min. In addition, the submitter states that the lyophilization has a 90% inactivation efficiency (p. 23).

MANUFACTURING/PROCESSING: Lyophilization of MCAN

Sites/Locations:

Manufacturing site not controlled by submitter and not yet identified (p. 1 and technical contact)

Days/yr: 2 days/yr

Basis: The technical contact estimates that the process would only happen once a year and take approximately 2 days (see contact report)

PROCESS DESCRIPTION:

The MCAN strain is sent to toll manufacturing site (likely Microbiologics, Inc. in St. Cloud, MN, per technical contact) for the lyophilization process. The exact processing operation will depend on the contract awardee for the material production. However, the expected processing steps would include culturing, resuspending in lyophilization matrix, aliquoting into individual samples, lyophilizing (or freeze-drying yeast containing the GEM), characterizing via colony growth on agar plates, and packaging into vials (p. 23-24).

ENVIRONMENTAL RELEASE SUMMARY

WATER/AIR/INCINERATION/LANDFILL:

From: Handling Powdered Yeast

Amount: 1.5×10^9 CFU/day, 2 days/yr

Basis: See Initial Review Engineering Report for details

$= 3.0 \times 10^8$ CFU/yr

$= 1.5 \times 10^8$ CFU/day

OTHER WATER: Negligible

Basis: See Initial Review Engineering Report for details

OTHER LANDFILL: Negligible

Basis: See Initial Review Engineering Report for details

OTHER INCINERATION: Not expected

Basis: See Initial Review Engineering Report for details

USE: Biological Detection Workflows

Number of Sites/Locations: up to 48 sites

Days/yr: 250 days/year

Basis: See Initial Review Engineering Report for details

PROCESS DESCRIPTION:

The submission and technical contact indicated that the MCAN will be used to for training personnel on biological detection of biothreat agents. For example, the GEM can be crushed into a powder and used in lieu of a suspicious material to safely challenge the biological assessment workflow. In addition, the MCAN may be used to validate microbial enumeration technologies (e.g., flow cytometer) (p. 23-24 and technical contact). To be conservative, RAD assesses 100% release of the MCAN for the training use.

ENVIRONMENTAL RELEASE SUMMARY

Submission does not estimate release amounts but provides some information about water, air, and solid waste releases. Technical contact stated that all waste is heat inactivated prior to disposal with either an autoclave or bleach. In addition, the lyophilization and rehydration will decrease the viability of the CFUs to less than 10% (p. 23). RAD used the GS methodology to estimate the releases from the downstream uses. RAD based releases on the trainings (estimated to be 70% of the PV) since the releases and exposures are more conservative than the biological instrument application (~30% of PV)

WATER/AIR/INCINERATION/LANDFILL

1) From: Training Release

Amount: 5.0×10^6 to 2.0×10^7 CFU/site-day CFU/site-day over 250 days/yr for 48 sites

Basis: See Initial Review Engineering Report for details

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CONSUMER and GENERAL POPULATION EXPOSURE

The Initial Review Engineering Report did not specify the media of release for the MCAN. In the absence of this information emissions were modeled as if they could be released to air or water.

Consumers

The MCAN will not be present in consumer products. Therefore, consumer exposure is not expected.

General Population

There is a potential for general population exposure as a result of releases to air and water from processing and use of the MCAN.

Inhalation Exposure

Releases to air from fugitive lyophilization emissions could occur at the processing site.

To estimate exposures from these sources the Gaussian algorithm described in Turner (1970) was used. The scenario assumes a release height of 10 meter; a 100 meter receptor distance from the source , a wind speed of 5.5 m/sec and a neutral atmospheric stability. The equation for a continuous release is as follows:

$$Conc = Q_{yr} \times 3.17 \times 10^{-12}$$

Since it is unlikely that, as a reasonable worst case, the wind blows in one direction. It is more reasonable to assume that the wind blows in one direction 25 percent of the time; therefore, the corrected equation is.

$$Conc = Q_{yr} \times 7.9 \times 10^{-13}$$

where:

Conc = Concentration in ambient air (CFU/m3)
Q yr = Release rate (CFU/yr)

Using the potential releases from the lyophilization process the concentration 100 meters downwind from the source would be :

$(3.0 \times 10^{9})) \times 7.9 \times 10^{-13}$ or < 1 CFU

The exposure from this concentration would be calculated as follows:

Exposure = C x IR x ET x AT

where:

C = concentration in ambient air (CFU/m3)
IR =Assumed inhalation rate of 1.1 m3/hr
ET =Exposure time is 24 hr/day
AT =Averaging time is 2 day yr

The estimated inhalation exposure resulting from this releases is negligible (<1 CFU/yr).

Releases to air from use of the MCAN could also occur. In the absence of additional information the releases from use modeled as above to estimate general population exposures.

The estimated inhalation exposure resulting from releases from use is 25 CFU/yr).

Drinking Water Ingestion Exposure

Estimates for drinking water exposure from releases of the MCAN from processing and use are given below.

If emissions from lyophilization were released to water, they would be treated at the local POTW in St. Cloud, MN (Permit MN0040878) and ultimately discharged to the Mississippi River (Reach 07010203010) which has a mean flow of 1.21×10^{4} MLD.

The surface water concentration of the MCAN from discharged waste on the day of release is generally calculated as

$$\text{Concentration (CFU/L)} = \frac{\text{Release (CFU/day)}}{\text{Surface water flow (MLD)}}$$

Thus, the estimated daily concentration of the MCAN in the receiving stream (Mississippi River) would be <1 CFU/L

In the event that drinking water was drawn near the discharge point without additional dilution, ingestion exposure is calculated for an individual drinking 2 L of this water per day as

Daily Drinking Water Exposure = (2 liters/day) x (surface water concentration).

Annual Drinking Water Exposure = (2 liters/day) x (days of release/year) x (surface water concentration).

Thus, the estimated drinking water exposure for the 2 day release would be < = 1 CFU

If emissions from use of the MCAN were released to water, they would be treated at the local POTW and ultimately discharged to surface water. Because the location of use sites is unknown, a generic mean flow rate for surface waters receiving POTW effluent was used as above in place of site specific flow data.

The estimated drinking water exposure for the 250 day release would be 80 CFU

REFERENCES

Jackson,E. 2018. Initial Review Engineering Report J-18-0044